THE EFFECT OF ALCOHOL ON THE CHOLINE REQUIREMENT

I. Changes in the Rat's Liver Following Prolonged Ingestion of Alcohol*

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Clinical evidence strongly suggests that there is a relationship between chronic alcoholism and the development of Laennec's cirrhosis. However, little is known about the underlying mechanisms involved. It is generally believed that the effect of alcohol on the liver is an indirect one related to a reduction in food consumption, and, hence, in the supply of lipotropic substances. This concept is based on the well known fact that malnutrition is a frequent complication of chronic alcoholism, and that animals deprived of choline may develop hepatic lesions closely resembling Laennec's cirrhosis. However, recent experimental studies indicate that alcohol may play a more direct role in this process.

Best and his associates (1) have presented evidence to show that alcohol increases the choline requirement by augmenting the caloric intake. These investigators found that rats maintained on a fluid intake of 15 per cent alcohol and a diet marginal in lipotropic activity developed fatty infiltration and fibrosis of the liver. Similar lesions were also observed in pair-fed controls given isocaloric equivalents of sucrose instead of alcohol, but not in pair-fed controls receiving neither sucrose nor alcohol supplements. Since the effects of alcohol and sucrose appeared to be identical, and since they were readily abolished by supplements of choline, methionine, or casein, it was concluded that the addition of alcohol and sucrose calories to a diet of marginal lipotropic activity had created a relative choline deficiency.

The results of the Best experiment, while consistent with the hypothesis that the choline requirement was a function of the caloric intake, did not exclude other possible interpretations, since it was not clearly established that the hepatic effects of alcohol were necessarily related either to an increase in the caloric intake or to an induced choline deficiency. In particular, it was not demonstrated that the effects of

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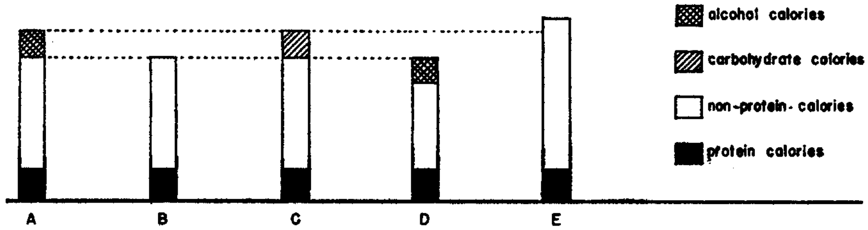
alcohol could be abolished by restricting dietary calories, or that alcohol was capable of inducing other signs of choline deficiency, such as renal necrosis.

It is the purpose of this report and the one to follow to present evidence which indicates that alcohol increases the choline requirement, but does not do so by augmenting the caloric intake.



TEXT-FIG. 1. Schematic representation of the distribution of dietary and alcohol calories.

Methods

Animals.—One hundred and thirty male Sprague-Dawley rats, weighing approximately 150 gm. each, were housed in individual cages in an air-conditioned room kept at 25°C. and a humidity of 50 to 55 per cent. The animals were divided into the following groups, whose distribution of dietary calories is illustrated in Text-fig. 1:—

A, basal diet and alcohol *ad libitum* (15 animals, 10 survivors).

AM, pair-fed with Group A and given the same amount of alcohol; however, the basal diet was supplemented with 0.5 per cent methionine (8 animals, 6 survivors).

AK, same as Group AM, except that the basal diet was supplemented with 0.5 per cent choline instead of methionine (7 animals, 7 survivors).

B, pair-fed with Group A but given no alcohol, so that it was not isocaloric with Group A (15 animals, 9 survivors).

C, pair-fed with Group A but given an isocaloric supplement of sucrose instead of alcohol, so that it remained isocaloric with Group A (15 animals, 12 survivors).

CM, same as Group C, except that the basal diet was supplemented with 0.5 per cent methionine (8 animals, 8 survivors).

CK, same as Group C, except that the basal diet was supplemented with 0.5 per cent choline (7 animals, 6 survivors).

D, pair-fed with Group A and given the same amount of alcohol; however, the total caloric intake was restricted to that of the non-alcoholic pair-fed controls in Group B; this was accomplished by subtracting sucrose from the basal diet equivalent in calories to the amount of alcohol consumed in Group A. Thus, these animals were identical with those in Group A with respect to their intake of alcohol and lipotropic substances, but received fewer calories (15 animals, 11 survivors).

DM, same as Group D, except that the basal diet was supplemented with 0.5 per cent methionine (8 animals, 7 survivors).

DK, same as Group D, except that the basal diet was supplemented with 0.5 per cent choline (7 animals, 6 survivors).

E, basal diet *ad libitum* without alcohol (25 animals, 21 survivors).

Feeding Technic.—Pair-feeding was done on an individual basis; *i.e.*, each rat in Group A was pair-fed with its own control in each of the other groups. In the case of the methionine- and choline-supplemented controls, however, only half of Group A was paired with each group. When an animal in Group A succumbed the dietary and alcoholic intake for its surviving controls was calculated on the basis of the average for the survivors in Group A.

The diet for Group A was weighed out in tared feeding cups at 3-day intervals. At the end of each period the cups were reweighed and the diet consumed calculated by difference. Additions and subtractions were then made as indicated above. For the sake of convenience in calculating the allowance for Group D rats, a carbohydrate-free diet was prepared which had a composition identical with that of the basal diet except for the absence of sucrose.

The basal diet employed had the following composition:—

Dietary constituents	Per cent	Calories	Per cent calories
Casein (Labco)*	12	48	9.5
Crisco	20	225	44.6
Corn oil*	5		
Sucrose	57.8	232	45.9
Cystine	0.2		
Salts IV	5		
Total	100	505	100

* Vitamin content per 100 gm. of diet:—

B_1	1 mg.	Inositol	20 mg.
B_2	1 "	Paraminobenzoic acid	20 "
B_6	1 "	Vitamin K	500 gamma
Nicotinic acid	10 "	Alpha-tocopherol acetate	10 mg.
Pantothenic acid	10 "	Vitamin D_2	10 gamma
Biotin	100 gamma	Vitamin A	3000 I.U.
Folic acid	100 "		

Alcohol Feeding.—Alcohol-fed rats received a 15 per cent aqueous dilution by volume of 95 per cent ethyl alcohol as their only source of drinking water. The solution was dispensed in Richter drinking tubes (2) which prevented significant losses by evaporation and leakage. 1 ml. of this solution was equivalent to 0.113 gm. of absolute alcohol and had a caloric value of 0.79. In calculating the isocaloric equivalents of sucrose to be added or subtracted in the control groups, alcohol was given its full value of 7 calories per gm., so that each milliliter of alcoholic drinking water was assumed to be equivalent to 0.2 gm. of sucrose. The reasons for not giving alcohol a value three-quarters of this amount, as in the experiments of Best *et al.* (1), have been discussed elsewhere (3). Measurements of alcohol consumption were made at 3-day intervals.

In general, the animals in each of the control groups consumed all of the calculated food and alcohol provided. If they did not the residue was mixed with the next ration. Nevertheless, there was some variation in the intakes, as is evident from the data recorded in Table I.

Chemical and Histological Technics.—During the course of the experiment all feces were collected and pooled by groups for biweekly nitrogen determinations by the Kjeldahl method.

Animals in Groups A and E were sacrificed by decapitation on the 223rd day, all others 3 days later. The liver was dissected free, dried by blotting, and weighed. Wedges of tissue were then excised from the right and left lobes and fixed in 10 per cent formalin. Paraffin-imbedded sections were stained with hematoxylin and eosin and a modification of the Masson trichrome stain. Frozen sections were stained with Sudan IV. Similar histological studies were carried out on the pancreas.

The remaining liver was reweighed and dried to constant weight in an air oven at 100°C.

TABLE I

Summary of Total Weight Gain and Mean Daily Intake of Food, Alcohol and Calories

Experimental groups	Survivors	Weight		Diet consumed	Alcohol consumed	Caloric intake			
		Initial	Gain			Dietary	Alcohol	Sucrose supplement	Total
		gm.	gm.	gm.	ml.*				
A Basal diet + alcohol *ad libitum*	10/15	145 ±22	254 ±58	9.6 ±1.4	12.5 ±2.2	48.2 ±7.1	9.9 ±1.8	—	58.1 ±5.5
AM Pair-fed diet + alcohol, +0.5 per cent methionine	6/8	130 ±17	253 ±44	8.4 ±1.2	11.8 ±2.4	42.1 ±6.2	9.3 ±1.9	—	51.4 ±7.9
AK Same as Group AM except + 0.5 per cent choline	7/7	168 ±12	224 ±35	8.9 ±0.8	12.4 ±1.3	44.8 ±4.4	9.8 ±1.0	—	54.6 ±4.1
B Pair-fed diet only	9/15	145 ±24	229 ±26	9.0 ±1.3	—	45.5 ±6.5	—	—	45.5 ±6.5
C Pair-fed diet + sucrose supplement (isocaloric with A)	12/15	154 ±27	224 ±38	9.0 ±1.2	—	45.5 ±6.3	—	9.7 ±1.8	55.3 ±7.3
CM Same as Group C, + 0.5 per cent methionine	8/8	131 ±16	260 ±52	8.8 ±1.4	—	44.5 ±6.8	—	±9.4 ±1.9	53.9 ±8.5
CK Same as Group C, + 0.5 per cent choline	6/7	169 ±14	233 ±34	9.1 ±1.0	—	45.9 ±5.2	—	10.4 ±1.6	56.3 ±5.7
D Pair-fed diet + alcohol, sucrose subtracted (isocaloric with Group B)	11/15	146 ±26	166 ±23	3.8‡ ±0.5	11.5 ±1.5	24.4 ±2.9	9.1 ±1.2	11.5§ ±2.0	44.4 ±6.9
DM Same as Group D, + 0.5 per cent methionine	7/8	129 ±18	208 ±20	4.0‡ ±0.6	12.1 ±2.5	25.5 ±4.1	9.5 ±1.9	11.4§ ±2.1	46.5 ±7.7
DK Same as Group D, + 0.5 per cent choline	6/7	170 ±12	159 ±30	3.9‡ ±0.3	12.5 ±1.7	25.7 ±2.9	9.9 ±1.4	11.4§ ±1.5	47.0 ±4.1
E Basal diet *ad libitum*	21/25	157 ±7	338 ±54	13.5 ±1.2	—	68.1 ±6.0	—	—	68.1 ±6.0

±, standard deviation.

Statistically significant differences: gain in weight: E > A (t = 3.73, p < 0.01), E > B (t = 7.28, p < 0.01), E > C (t = 7.0, p < 0.01), E > D (t = 12.4, p < 0.01), A > D (t = 4.35, p < 0.01), B > D (t = 5.53, p < 0.01), C > D (t = 4.45, p < 0.01); caloric intake: E > A (t = 4.57, p < 0.01), E > B (t = 8.58, p < 0.01), E > C (t = 5.19, p < 0.01), E > D (t = 9.61, p < 0.01), A > B (t = 4.32, p < 0.01), C > B (t = 3.08, p < 0.01), A > D (t = 4.83, p < 0.01), C > D (t = 3.50, p < 0.01).

* 15 per cent aqueous solution of 95 per cent ethyl alcohol.

‡ Special sucrose-free basal diet; provided same amount of protein, fat, and vitamins as in Group A.

§ Sucrose added to bring caloric intake up to that of Group B.

Total lipids were then determined by the method of the Association of Official Agricultural Chemists (A.O.A.C.) (4) using a Nolan extractor (5).

During the course of the experiment a number of animals died of intercurrent pulmonary infections. These were excluded from consideration in the data presented. However, the hepatic changes observed did not differ significantly from those in the survivors of each group.

RESULTS

Growth and Food Consumption.—(Table I.) All groups gained weight at a satisfactory rate, although not as rapidly as those maintained on a 22 per cent casein diet in a previous experiment (3). However, they achieved a greater weight than animals receiving the 18 per cent casein-gelatin-zein mixture employed by Best *et al.* (1).

Alcohol ingestion resulted in a decrease in food consumption, as is evident from a comparison of the animals in Group A with the *ad libitum* fed controls in Group E, and this was accompanied by a reduction in the total caloric intake, despite the addition of alcohol calories. As a consequence, the alcohol-fed animals gained less weight.

The alcohol-fed animals in Group D, whose caloric intake was restricted, gained the least weight. Since they received approximately the same number of calories as the non-alcoholic controls in Group B, the suggestion of Mitchell (6) that alcohol is less effective than carbohydrate in supporting growth appeared to be confirmed. However, this difference was not evident when the alcohol-fed animals in Group A were compared with their isocaloric, sucrose-supplemented, pair-fed controls in Group C. The significance of this observation is still in doubt, since neither the alcohol nor sucrose supplements in Groups A and C produced significantly greater growth than occurred in the unsupplemented animals of Group B. Perhaps these inconsistencies can be related to the fact that no account was taken of the animals that died in each group. However, it may be pointed out that essentially the same results were obtained in a previously reported experiment (3) in which only one animal died.

Alcohol Consumption.—(Table I). The average intake of 15 per cent alcohol in Group A was 12.5 ml. daily, an amount considerably less than that consumed in the Best experiment (18.3 ml.) (1). Moreover, alcohol provided a smaller fraction of the total calories ingested (17 as compared to 22 per cent). Although these differences may have been related to individual, environmental, or dietary factors, a question may be raised concerning the validity of the high values reported from Toronto, in view of the type of alcohol feeder employed. These investigators used an inverted bottle with a terminally constricted curved glass delivery tube, an apparatus which in this laboratory has been found to be unreliable in that it permits considerable and unpredictable losses of alcohol through leakage.

Liver Lipids.—(Table II). The basal diet was slightly deficient in lipotropic activity, as in the Best experiment (1), so that the hepatic lipids in the *ad libitum*-fed controls of Group E were significantly higher than those in animals

TABLE II

Hepatic Lipids and Fibrosis

Experimental groups	No. of rats	Hepatic lipids	Histological changes						
			Fatty infiltration‡						Fibrosis§
			0	±	1+	2+	3+	4+	
		*gm. per cent**							
A Basal diet + alcohol *ad libitum*	10	7.6 ± 8.1	1	3	3	1	1	1	2/10
AM Pair-fed diet + alcohol, + 0.5 per cent methionine	6	3.0 ± 1.0	0	6	0	0	0	0	0/6
AK Same as Group AM except 0.5 per cent choline	7	1.4 ± 1.3	4	2	1	0	0	0	0/7
B Pair-fed diet only	9	5.7 ± 2.1	0	3	4	2	0	0	1/9
C Pair-fed diet + sucrose supplement (isocaloric with A)	12	16.6 ± 12.3	1	3	2	1	1	4	5/12
CM Same as Group C, + 0.5 per cent methionine	8	3.4 ± 0.5	7	0	0	0	1	0	0/8
CK Same as Group C, + 0.5 per cent choline	6	2.6 ± 0.8	5	1	0	0	0	0	0/6
D Pair-fed diet + alcohol, sucrose subtracted (isocaloric with Group B)	11	14.1 ± 8.2	1	4	0	1	4	1	6/11
DM Same as Group D, + 0.5 per cent methionine	7	2.9 ± 1.3	3	3	1	0	0	0	0/7
DK Same as Group D, + 0.5 per cent choline	6	2.5 ± 1.4	5	0	1	0	0	0	0/6
E Basal diet *ad libitum*	21	11.1 ± 5.8	4	6	2	4	3	0	3/19

Statistically significant differences: hepatic lipids: D > B ($t = 3.1$, $p < 0.01$), C > B ($t = 2.90$, $p < 0.01$), E > B ($t = 3.65$, $p < 0.01$), A > AK ($t = 2.25$, $p < 0.05$), C > CM ($t = 3.57$, $p < 0.01$), C > CK ($t = 3.75$, $p < 0.01$), D > DM ($t = 4.23$, $p < 0.01$), D > DK ($t = 4.34$, $p < 0.01$); fibrosis: D > B ($\chi^2 = 6.89$, $p < 0.01$), D > E ($\chi^2 = 3.86$, $p = 0.05$), C > B ($\chi^2 = 3.31$, $p < 0.05$).

* Based on wet weight of liver; ± = standard deviation.

‡ Based on Sudan-stained frozen sections; ± = 5 per cent or less of cells contained fat droplets, 1+ = 5–25 per cent, 2+ = 25–50 per cent, 3+ = 50–75 per cent, 4+ = >75 per cent. Two blocks lost in Group E.

§ Based on Masson-stained sections.

maintained on a 22 per cent casein diet (3). Although the amount of fat did not differ significantly from that reported by Best *et al.* (1) (10.0 per cent), much higher values might have been anticipated since the basal diet in the present experiment (*a*), was administered for a longer period of time (223 as compared to 177 days), (*b*), had a higher proportion of fat (25 as compared to 12 per cent), and (*c*), had a lower estimated content of lipotropic substances

(360 mg. of methionine as compared to 420 mg. of methionine plus 50 mg. of choline per 100 gm. of diet). However, the Best diet contained only 10 per cent casein and 8 per cent of a mixture of gelatin and zein, proteins known to be deficient in a number of essential amino acids other than methionine. Since there is suggestive evidence to indicate that the lipotropic activity of a diet may be related to its content of such amino acids (7), conceivably the activity of the Best diet was actually lower than ours. The fact that it produced considerably more hepatic fat in animals corresponding to Groups A, B, and C would also point in this direction.

In contrast to the results of Best *et al.* (1), neither alcohol nor sucrose supplements in Groups A, C, and D produced significantly greater fatty infiltration than occurred in the *ad libitum* fed controls of Group E. However, when the former are compared with their pair-fed controls in Group B, it becomes apparent that both alcohol and sucrose supplements produced an increase in the fat content of the liver. Especially noteworthy is the fact that Group D animals, whose caloric intake was restricted, had significantly more hepatic fat than their isocaloric controls in Group B, indicating that the effect of alcohol on the liver could not have been due to an increase in the caloric intake. Although alcohol also produced an apparent increase in the lipids in Group A rats, whose caloric intake exceeded that of Group B, the difference was not statistically significant. However, the number of animals studied may have been too small to establish a significant difference, and, indeed, evidence will be presented in the following paper to show that alcohol increases the choline requirement even when calories are not restricted. The failure to demonstrate any differences between the *ad libitum*-fed animals in Group E and those receiving alcohol or sucrose supplements in Groups A, C, and D can probably be related to the fact that the former ate more and grew more rapidly, thereby increasing their choline requirement and obscuring the effects of alcohol and sucrose. The fact that the hepatic lipids in Group E were significantly higher than those in Group B, in which the dietary intake and rate of growth were limited, lends weight to this interpretation.

The addition of choline or methionine to the basal diet abolished the effects of alcohol and sucrose supplements, as indicated by the marked lowering of the hepatic liqids in Groups AM, AK, CM, CK, DM, and DK. This suggests that however alcohol and sucrose produced a fatty liver they did not do so by interfering with the absorption, utilization, or synthesis of choline.

Although alcohol and sucrose supplements appeared to be identical, in that both produced a type of fatty infiltration which could be prevented by adding lipotropic substances to the basal diet, it does not follow that their modes of action in the liver were necessarily the same. Conceivably the sucrose effect was related to an increase in the caloric intake, although even this was not established, but certainly the evidence clearly indicates that the caloric factor was not important in the case of alcohol. As far as the interpretation of the increase in hepatic lipids is concerned, the data are consistent with the hypothesis that alcohol induced a relative choline deficiency, but they are certainly not conclusive. However, more convincing evidence of such a deficiency will be presented in the following paper.

It will be noted that there were marked individual differences in the fat content of the liver in all groups except those supplemented with choline or methionine. These could not be related to individual variations in food intake, alcohol consumption or the rate of growth, and, thus, remain unexplained.

Histological Changes in the Liver.—The amount of stainable fat was graded 0 to 4+ (Table II). In general, the results confirmed the chemical findings, but the differences between the various groups were less distinct. A comparison of the fat determinations by both methods

TABLE III

Comparison of Chemical and Histological Estimates of Hepatic Lipids

Histological grading*	Chemical analysis, *gm. per cent of wet weight*							
	0.5–4.0	4.1–8.0	8.1–12.0	12.1–16.0	16.1–20.0	20.1–24.0	24.1–28.0	>28.0
0	24	4	2	0	0	0	0	0
±	19	9	0	1	0	1‡	0	0
1+	2	7	3	1	0	0	0	0
2+	2	3	3	3	0	1	0	0
3+	1	0	0	3	1	2	1	2
4+	0	1	0	0	0	0	2	3

* Based on Sudan-stained frozen sections, see footnote Table II for method of grading.

‡ Possibly an error in labelling; hematoxylin and eosin section showed 4+ fat; tissue discarded so that frozen sections could not be checked.

(Table III) makes it clear that the amount of stainable fat was only a crude index of the actual lipid content of the liver as a whole, suggesting that the distribution of fat was not uniform and that the histological technic was subject to sampling errors.

In hematoxylin and eosin-stained sections the fat appeared as large vacuoles filling or even distending individual parenchymal cells. However, Sudan-stained sections revealed, in addition, numerous small fat droplets of much wider distribution. The fat tended to be most heavily deposited in the central zones of the lobules, but in approximately 20 per cent of the sections it was periportal in distribution. A similar variability in the localization of fat was observed in a control series of weanling rats maintained on a low choline diet, although in this instance the peripheral localization of fat was somewhat less common.

Some degree of hepatic fibrosis was encountered in all groups except those receiving choline or methionine (Table II), which was to be expected in view of the relatively low lipotropic activity of the basal diet. However, the number and extent of such lesions were greatest in the alcohol and sucrose-supplemented animals of Groups D and C, although in no instance were they as extensive as those illustrated in Best's paper (1). In most cases the connective tissue proliferation was confined to the periportal and central areas with relatively few slender extensions into the surrounding parenchyma. However, a few animals showed thin fibrous bands connecting adjacent portal zones and central veins giving rise to pseudolobules, as illustrated in Fig. 1. Although the occurrence of hepatic fibrosis following prolonged fatty infiltration is consistent with a chronic choline deficiency, it cannot be considered pathognomonic, since a similar sequence of events is known to occur in other conditions (8).

Since chronic alcoholism (9) and certain nutritional disturbances (10, 11) are often associated with pancreatic lesions, some of which may give rise to fatty infiltration of the liver, the pancreas was examined with considerable care. Except for one instance of chronic pancreatitis in a control animal in Group E, none of the glands exhibited significant changes.

TABLE IV

Fecal Nitrogen Excretion

Experimental Groups		Fecal nitrogen	Dietary nitrogen	Nitrogen lost in feces
		*mg. per rat per day**	*mg. per rat per day‡*	*per cent*
A	Basal diet + alcohol *ad libitum*	5.6 ± 2.4	186 ± 27	3.0
AM	Pair-fed diet + alcohol, + 0.5 per cent methionine	8.3 ± 3.3	167 ± 24	5.0
AK	Same as Group AM except + 0.5 per cent choline	8.0 ± 2.9	178 ± 16	4.5
B	Pair-fed diet only	12.5 ± 3.7	175 ± 25	7.2
C	Pair-fed diet + sucrose supplement (isocaloric with A)	6.3 ± 2.5	175 ± 23	3.6
CM	Same as Group C, + 0.5 per cent methionine	8.0 ± 3.3	175 ± 28	4.6
CK	Same as Group C, + 0.5 per cent choline	10.1 ± 4.8	182 ± 20	5.5
D	Pair-fed diet + alcohol, sucrose subtracted (isocaloric with Group B)	6.6 ± 3.2	174 ± 23	3.8
DM	Same as Group D, + 0.5 per cent methionine	6.0 ± 2.7	183 ± 27	3.3
DK	Same as Group D, + 0.5 per cent choline	8.0 ± 3.7	179 ± 14	4.5
E	Basal diet *ad libitum*	11.9 ± 4.3	262 ± 23	4.5

* Calculated on basis of analysis of pooled specimens obtained during 15 collection periods totalling 223 days.

‡ Estimated from dietary intake, assuming 1 gm. of casein contained 160 mg. of N, 1 gm. methionine 94 mg. N, and 1 gm. choline 116 mg. N.

Fecal Nitrogen Excretion.—As indicated by the data in Table IV animals receiving alcohol lost no more nitrogen in their feces than their pair-fed controls. It is reasonable to assume, therefore, that the effects of alcohol on the liver were not related to any impairment of protein digestion or absorption.

SUMMARY

Rats maintained for a period of 7 months on a fluid intake of 15 per cent alcohol and a diet marginal in lipotropic activity developed fatty infiltration and mild fibrosis of the liver. Similar changes were observed in pair-fed controls given an isocaloric equivalent of sucrose instead of alcohol, but not in pair-fed controls receiving neither alcohol nor sucrose supplements. To ex-

clude the possibility that the alcohol effect was related to an augmentation of the caloric intake, a third group of controls was given the same amount of alcohol, but a limited number of calories. This was accomplished by subtracting from the basal diet an amount of sucrose equivalent in calories to the alcohol consumed. Under these conditions the hepatic changes following alcohol ingestion appeared to be enhanced. Choline or methionine, on the other hand, abolished the effects of both alcohol and sucrose supplements. There was no increase in fecal nitrogen excretion following alcohol ingestion, and no histological changes were observed in the pancreas.

These results are consistent with the hypothesis that alcohol increases the choline requirement of the rat, but do not support the contention that this effect is the consequence of an augmented caloric intake. Further studies are needed to establish conclusively the relationship between alcohol ingestion and the choline requirement, and to elucidate the mechanisms involved.

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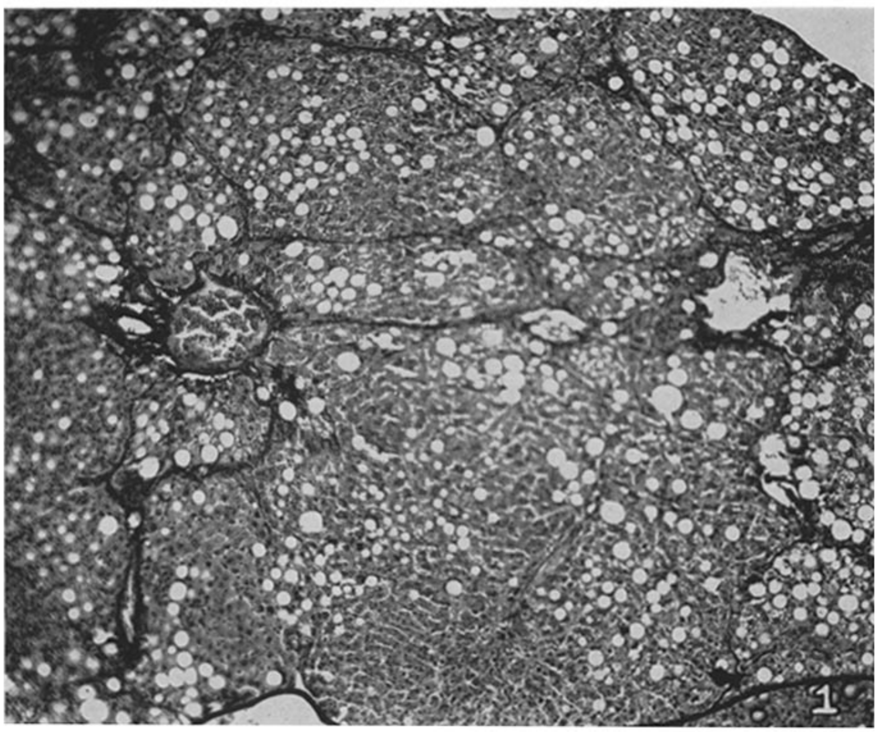
EXPLANATION OF PLATE 54

FIG. 1. Fatty infiltration and fibrosis of the liver in a Group D rat maintained on alcohol for a period of 7 months (Masson stain).



(Klatskin *et al.*: Alcohol and choline requirement. I)